



KINETIC STUDY OF THE FERMENTATION OF SOURSOP JUICE USING *SACCHAROMYCES CEREVISIAE* WITH EFFECT OF SUCCINIC ACID

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ABSTRACT

The fermentation of soursop juice as substrates by *Saccharomyces cerevisiae* (yeast) was investigated to obtain certain useful kinetic parameters. These were achieved by the determination of the effects of temperature, yeast, pH, substrate and inhibitor on the rate of carbon (IV) oxide (CO₂) production. The extent of inhibition was examined by the addition of succinic acid. The results indicate that the rate of fermentation increased in proportion with temperature (optimum 36°C), substrate (optimum 50%v/v) and succinic acid (optimum 30%v/v) up to a limit and decreased. This suggests that the reaction takes place in two steps (equilibrium and decomposition steps). The kinetic parameters examined are: maximum velocity of reaction, V_{max} for substrate, $2.58 \times 10^2 \text{ M}^1 \text{ min}^{-1}$ and succinic acid, $1.00 \times 10^2 \text{ M}^1 \text{ min}^{-1}$. Catalytic constant, k_2 ; for substrate, $8 \times 10^{-2} \text{ min}^{-1}$ and succinic acid, $6.0 \times 10^{-2} \text{ min}^{-1}$. Overall rate constant k , for substrate, 1.79×10^2 and succinic acid, 1.09×10^2 . The order of initial reaction is first order for substrate and the additive. The dissociation constants of enzyme-substrate complexes, k_s are 1.64×10^2 and succinic acid, 2.40×10^{-1} . The Michaelis constant, k_m for the substrate, $1.64 \times 10^2 \text{ M}$ and succinic acid, 2.50×10^{-1} . The specific activity of enzyme for the substrate is 1×10^{-1} and succinic acid, 2×10^{-2} . Finally, the type of inhibition was found to be uncompetitive from the extrapolated Lineweaver-Burk plots and the suggested inhibitor did not bring about marked decrease in the rate of fermentation as expected. **KEY WORDS:** soursop, fermentation, kinetic study, *saccharomyces*, kinetic study, succinic acid.

Keywords and Phrases: Kinetic study, fermentation, soursop and succinic acid.

INTRODUCTION

Fermentation is the process of producing alcohol through breakdown of carbohydrate substrates such as sugar, starch and cellulosic materials by the action of microorganisms or enzymes. During fermentation the substrate is utilized in the production of alcohol as well as in the growth of the microorganisms. In this process starch is broken down into fermentable sugars by fungal enzymes such as alpha-Amylase and Gluco-amylase to facilitate fermentation by mainly *Saccharomyces* species. Fermentation could occur under anaerobic or aerobic conditions and yields lactate, acetic acid, ethanol, carbon dioxide or some other simple products (Sanlier, and Gokcen, 2019).

Saccharomyces cerevisiae is a microorganism with a general application in food manufacturing. It is very important in many food fermentations such as bread, wine, beer



and table olives, but it also causes spoilage by re-fermentation after packaging regarding table olives, bottling of wines, beverages and fruit concentrates (Copeland, 2000). The typical fermentation is anaerobic, but *Saccharomyces cerevisiae* is a facultative anaerobe and can respire on low sugar concentrations while using respiratory substrates (Johansson, 2013).

Kinetics in general is concerned with reaction rates. Kinetics simply suggests a primary concern for the rates of commercially practiced reactions and particularly with the effects of process variables on them. In order to effectively analyze and subsequently optimize a biological process, the kinetics of the process need to be understood and quantified. The use of kinetic models to describe the behavior of biological system has been acknowledged to be important because it can reduce the number of experiments needed to eliminate extreme possibilities and provide mathematical expressions that can quantitatively describe the mechanism of the process as required for optimization and control (Winzor, 2006).

The growth of microorganisms during bioconversion is a complex process. The Monod, Michealis and Line-weaver Bulk equations are usually used to relate specific growth rate to the concentration of the limiting substrate (Leksawasdi, 2001). Their equations are well suited for fermentation processes where the growth of fermenting organism is not inhibited by toxic substances. It is known that microbial activity during ethanol fermentation is affected by certain factors namely, cell death, substrate limitation, substrate inhibition and product inhibition. (Palmer, 2000). None of the models in the literature account for the effect of these factors at the same time. The model of Abba et al., (1988), only account for the effect of product inhibition. For a kinetic model to appropriately represent microbial activity during ethanol fermentation, it should account for the effect of all four factors even though this is the desired outcome. It is not realistic to expect that any kinetic model to be able to correctly represent real process situations.

The purpose of this study is to investigate the influence of succinic acid inhibitor on ethanol production (Malinowski, 2001).

Soursop (*Annona muricata*) is well-known for its pleasant, highly aromatic juicy flesh and distinctive flavour. The soursop fruit has a relatively short shelf life because it gets bruised easily during handling and transportation. It is also difficult to consume fresh due to its mushy flesh. To prolong its shelf life, it may be processed into other forms like puree, juices, nectars syrups, concentrates, jams jellies, ice creams, powders, fruit bars and flakes. Although the soursop juice is popularly produced for its convenience to be consumed, the yield of its juice is little due to its cotton pulpy flesh (Liaw, 2002).

Materials and method

Burette, clamp and retort stand, flasks, filter cloth, fermentation vessels, measuring cylinder, pH meter, rubber tubes, refrigerator, stirring rod, stop watch, thermometer, weighing balance, and water bath. Soursop fruits were purchased from Ekpoma market, Esan West L.G.A. Edo State, Nigeria. pH meter pocket-sized, Hanna product (Hospito



Mart Complex), was standardized with appropriate buffer solution at pH 4. Yeast (*Saccharomyces cerevisiae*), was supplied by Vahine professional, Mc cormick, France SAS and was used as received. sulphuric acid, sodium hydroxide, hydrochloric acid, phenolphthalein and succinic acid were purchased from reputable suppliers (Hospito Mart Complex and analyst grades) and used without further purification. The analysis was carried out at Chemistry Laboratory, Samuel Adegboyega University Ogoja, Edo State Nigeria.

Extraction of Soursop

Fresh, healthy and mature soursop fruits of various sizes were collected from Ekpoma, Esan West Local Government area of Edo state. Five fully ripe soursop fruits with an average weight of 2.8154kg (Setra BL.2008 balance) were washed thoroughly with distilled water and surface sterilized with 70% ethanol. The fruits were peeled with sterile knife to remove the skin and then deseeded. The juice was then manually squeezed out with a muslin cloth by hand and preserved in a refrigerator (Thermocool). 500cm³ of juice was obtained from 2.8154kg of soursop. The juice was filtered and treated with a 3% Sodium metabisulphite, (Na₂S₂O₅) to inhibit the growth of any undesirable microorganism such as acetic acid bacteria, wild yeast and mould (Copeland, 2000). Thereafter, the required quantity (20-80%) of juice was transferred into the fermentation vessels (fermenters). Effect of succinic acid inhibitor on fermentation kinetics and parameters were examined.

Experimental Procedure

The fermentation vessels were sterilized with a 3% solution of sodium metabisulphite for 5 minutes. A litre (1000cm³) of juice was properly conditioned and was brought to the required pH with either 0.1M HCl or 0.1M NaOH. Seven fermenters (polyethylene terephthalate bottles 75cl) containing substrate were connected with tubes to evolve the produced carbon (IV) oxide and prepared for each, seven reaction times ranging from 30 -210minutes at 30minutes intervals of time. Yeast was added to each of the fermenter. The substrate and the yeast were properly mixed by shaking and the yeast was allowed to activate for 20minutes. The escape of CO₂ was prevented by sealing the air inlet with a cresol-perfumed jelly. The CO₂ produced in each sealed fermenter was collected in water and measured by titration, with 0.1M NaOH using phenolphthalein indicator. The rate of fermentation was measured as the volume of CO₂ produced at 30 minutes intervals of time. The effect of temperature on fermentation was determined by keeping other factors such as substrate concentration, pH of the juice, yeast concentration and fermentation time constant. The temperature was varied between 30-42°C, using a thermostat water bath. In determining the effect of substrate concentration on fermentation, all other factors such as temperature, pH, yeast concentration, and fermentation time were kept constant. The substrate concentration was varied between 20–80(v/v). In determining the effect of pH on fermentation, factors like substrate concentration, temperature, fermentation time and



yeast concentration were kept constant. The pH of the juice was varied between 3.0-6.0 by the addition of 0.1M H₂SO₄ solution or 0.1M NaOH solution to the required pH value and measured by a pH meter. The effect of yeast concentration on fermentation was determined by varying yeast concentration between 1-7(w/v). The rate of fermentation was measured as the volume of CO₂ produced at 30 minutes intervals of time. The optimum substrate and yeast concentrations as well as pH and temperature required for the fermentation, were determined from the maximum heights achieved from the plot of the CO₂ produced versus time curves, measurements were in triplicates.

Determination of effect of Inhibitor

The effect of inhibitor on the rate of fermentation was determined by varying substrate concentration between 20-80%v/v. For each volume of substrate, 1-10ml of 0.1M solution of inhibitor (succinic acid) was added.

Determination of Kinetic Parameters: V_{max} , K_m , k_2 , k , n and K_s

For the kinetic parameters, modified Michaelis-Menten equation by Lineweaver-Bulk and Briggs- Halden for a single substrate-enzyme catalyzed reaction was used. The maximum velocity (V_{max}) and Michaelis constant (K_m) were obtained from the slope and intercept of the graph of reciprocal velocity versus substrate concentration using $1/v_o = k_m/V_{max} [S] + 1/V_{max}$. For the catalytic constant (k_2) and dissociation constant (k_s) yeast was varied between 1-7grams. For 1g substrate was varied between 20-80ml and time 30-210minutes at 30minutes interval. The procedure was repeated for 2-7g respectively. The volume of CO₂ produced was plotted against time to determine rate of fermentation. The maximum rate for yeast gram was plotted against yeast concentration and k_2 was obtained from the slope of the graph ($V_{max} = k_2[E_o]$) while k_s was determined by substitution using the equation $1/v_o = k_s/k_2[E_o]1/[S] + 1/k_2[E_o]$; $K_s = k_2[E_o]$. The order of the reaction (n) and rate constant (k) were determined using differential method: ($\log v = n \log[A] + \log k$)

RESULTS

The results of the effect of temperature, substrate concentration, pH, yeast concentration, (*saccharomyces cerevisiae*) inhibitor (succinic acid) on the fermentation of soursop juice are presented in Tables 1-16 and on Figures 1-20



Table 1: Data Of The Variation of Volume of CO₂ Produced With Time at Different Temperatures And Rate Of Fermentation Of Soursop Juice Using 50%(V/V) Substrate, Yeast 1.0%(w/v) and pH 5.0

Temperature (°C)							
Volume of CO ₂ produced (cm ³)							
Time(min)	30	32	34	36	38	40	42
30	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
60	1.2±0.1	1.2±0.3	1.4±0.1	1.5±0.1	1.2±0.1	1.4±0.4	1.2±0.2
90	1.7±0.2	1.7±0.5	1.5±0.2	2.0±0.2	1.7±0.2	1.7±0.3	1.4±0.2
120	2.0±0.0	2.0±0.3	2.0±0.1	3.2±0.2	2.0±0.2	1.8±0.3	2.0±0.3
150	2.2±0.3	2.2±0.6	2.2±0.2	3.8±0.1	2.2±0.2	2.0±0.7	2.2±0.1
180	2.7±0.1	2.5±0.1	3.7±0.3	4.7±0.3	3.2±0.1	2.2±0.4	2.5±0.1
210	3.7±0.5	3.0±0.1	4.0±0.1	5.2±0.4	4.0±0.3	3.5±0.2	2.7±0.1
Rate (molmin ⁻¹)	32.6	35.6	42.5	72.7	50.2	47.6	36.2

TABLE 2: Effect Of pH on the Rate of Fermentation of Soursop Juice using 50% (V/V) Substrate, 1.0% (W/V) Yeast, at 30°C

pH							
Volume of CO ₂ produced (cm ³)							
Time(min)	3.0	3.5	4.0	4.5	5.0	5.5	6.0
30	1.0±0.1	1.0±0.2	1.0±0.1	5.0±0.2	2.0±0.1	4.0±0.1	3.0±0.2
60	2.2±0.1	1.2±0.1	2.0±0.2	5.7±0.3	3.0±0.2	4.2±0.2	5.1±0.1
90	2.7±0.4	2.0±0.1	4.0±0.1	6.0±0.3	3.5±0.2	5.0±0.2	5.7±0.2
120	3.2±0.2	2.7±0.1	5.0±0.2	6.2±0.2	5.5±0.3	5.2±0.4	7.7±0.3
150	5.0±0.2	6.0±0.3	6.7±0.2	7.0±0.2	5.7±0.3	5.5±0.0	8.0±0.2
180	6.4±0.1	8.2±0.1	8.0±0.3	8.2±0.4	8.0±0.1	6.5±0.1	9.7±0.1
210	7.1±0.1	9.1±0.2	8.2±0.6	9.5±0.1	9.2±0.1	7.7±0.2	9.5±0.4
Rate (molmin ⁻¹)	2.90	3.30	3.90	4.40	4.70	4.00	3.90



Table 3: Data Of The Variation Of Volume Of CO₂ Produced With Time At Different Substrate Concentrations Of Soursop Juice Using 1.0 %(W/V) Yeast, At 30°C And pH 5.0

Substrate concentration%(v/v)							
Volume of CO ₂ produced (cm ³)							
Time(min)	20	30	40	50	60	70	80
30	1.0±1.6	1.4±1.6	2.8±0.5	1.2±1.1	1.1±2.0	3.1±1.3	3.1±1.4
60	2.7±0.4	1.7±1.3	3.5±1.9	1.4±0.9	1.5±0.3	3.2±1.2	3.7±1.2
90	3.1±0.5	2.7±1.5	4.8±0.4	1.8±0.2	1.7±1.2	4.7±0.1	5.0±0.3
120	4.8±2.1	4.4±1.4	5.2±0.3	2.2±1.0	2.0±0.4	6.0±0.1	5.2±1.9
150	5.5±2.0	4.5±2.2	6.0±0.1	2.5±1.2	7.0±0.1	6.1±0.1	6.0±0.1
180	6.1±2.4	5.7±2.2	6.2±0.1	2.8±0.1	7.7±0.1	6.7±0.1	7.2±0.8
210	7.5±1.9	8.5±0.3	7.2±0.1	3.5±0.4	8.7±1.3	7.0±0.1	7.5±0.2
Rate(molmin ⁻¹)	39.5	85.5	106.7	126.4	104.2	89.5	86.9

Table 4: Effect Of Yeast Concentration on Rate of Fermentation of Soursop Juice Using 50% (V/V) Substrate, at 30°C, and pH 5.0

Yeast concentration (grams)							
Volume of CO ₂ produced (cm ³)							
Time(min)	1	2	3	4	5	6	7
30	1.0±0.1	2.0±0.2	3.0±0.1	1.0±0.2	2.5±0.1	1.0±0.3	2.0±0.2
60	1.1±0.2	2.5±0.1	3.2±0.2	3.0±0.1	3.0±0.1	3.2±0.3	3.0±0.2
90	2.0±0.1	3.0±0.2	4.2±0.2	4.2±0.2	3.4±0.2	4.0±0.2	4.0±0.1
120	2.4±0.2	3.2±0.2	4.4±0.1	4.5±0.2	4.0±0.2	4.2±0.1	4.2±0.3
150	2.7±0.3	4.0±0.1	4.7±0.1	4.7±0.1	4.2±0.1	5.0±0.1	4.7±0.3
180	3.2±0.1	4.2±0.1	5.2±0.2	5.0±0.2	5.7±0.1	5.7±0.1	6.0±0.4
210	4.2±0.2	4.5±0.1	6.0±0.2	6.0±0.2	6.4±0.1	7.7±0.	7.0±0.2
Rate(molmin ⁻¹)	1.40	1.80	2.50	3.70	3.00	2.50	2.10

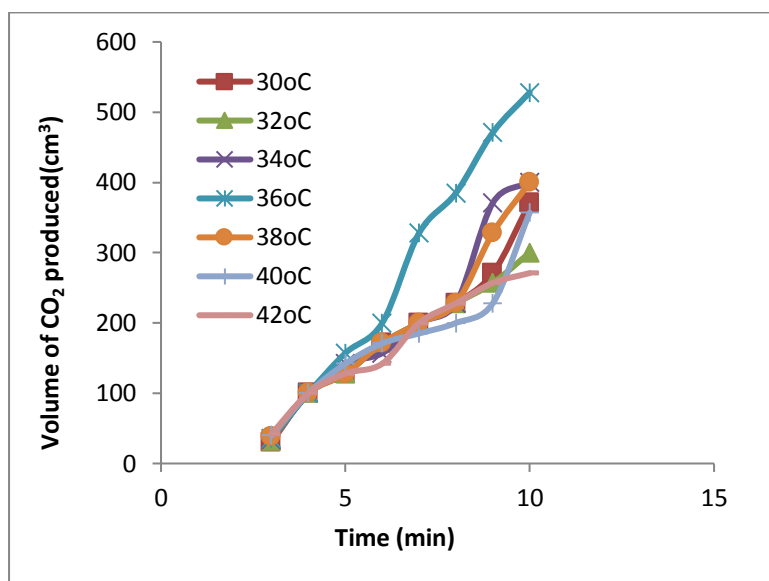


Fig.1: Variation Of Volume of CO₂ Production With Time At Different Temperatures Of Fermentation Of Soursop Juice Using 50% (V/V) Substrate, Yeast 1.0% (w/v), and pH 5.0

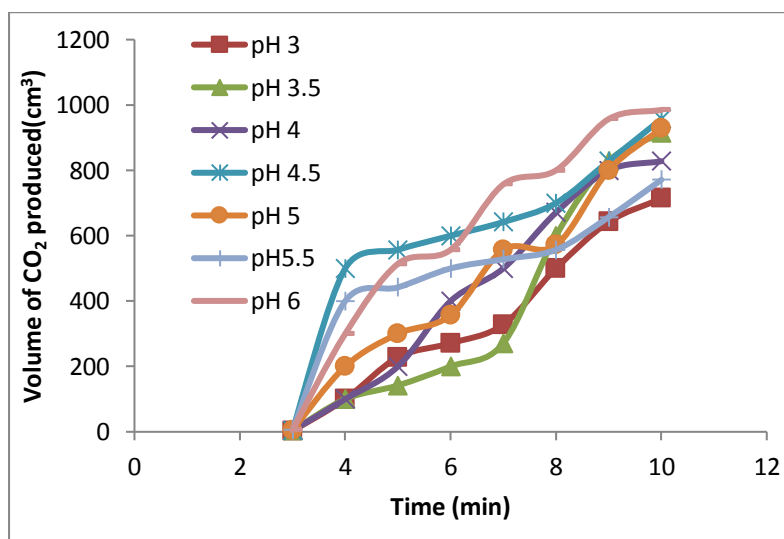


Fig.2: Variation of Volume of CO₂ Production with time at different pH Of Fermentation of Soursop Juice Using 50% (v/v) Substrate, 1.0 %(W/V) Yeast, at 30°C

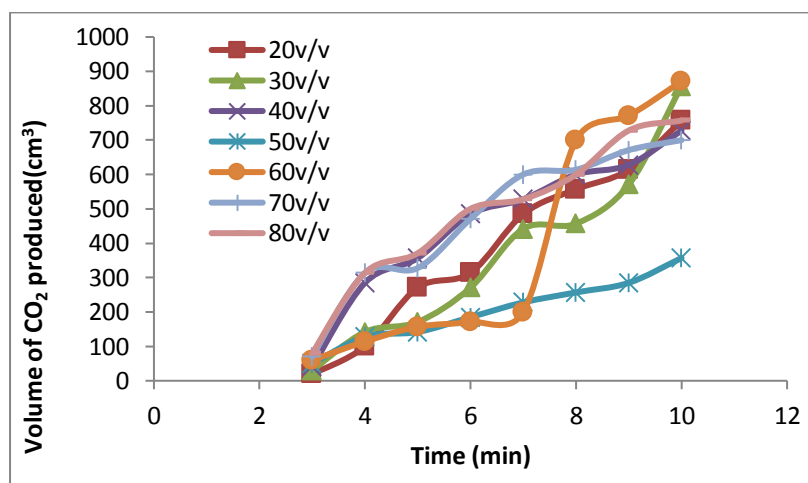


Fig.3: Variation of volume of CO₂ production with time at different substrate concentrations of fermentation of soursop juice using 1.0% (w/v) yeast, at 30°C and pH 5.0

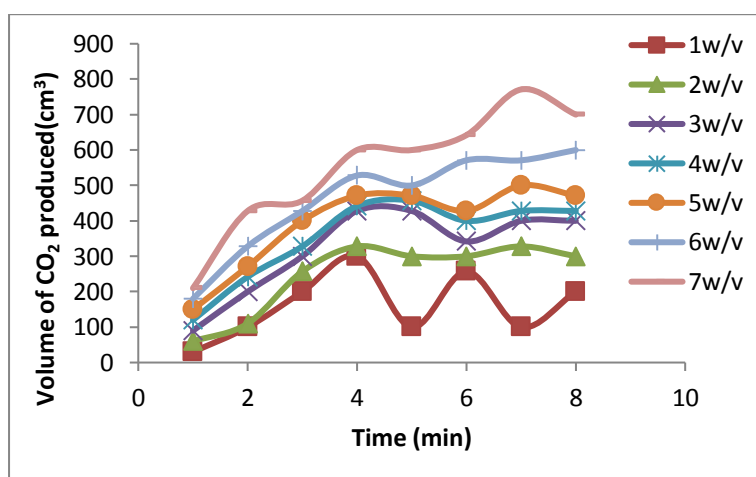


Fig.4: Variation of volume of CO₂ production with time at different yeast concentrations of fermentation of soursop juice using 50% (v/v) substrate, at 30°C, and pH 5.0



Table 5: Data of the rate of fermentation at different substrate concentration, temperature, pH and yeast concentration of soursop juice

RATE OF FERMENTATION(mol l^{-1})							
TEMPERATURE	32.6	35.6	42.5	72.7	50.2	47.6	36.2
pH	2.90	3.30	3.90	4.40	4.70	4.00	3.90
SUBSTRATE	39.5	85.5	106.7	126.4	104.2	89.5	86.9
YEAST	1.40	1.80	2.50	3.70	3.00	2.50	2.10

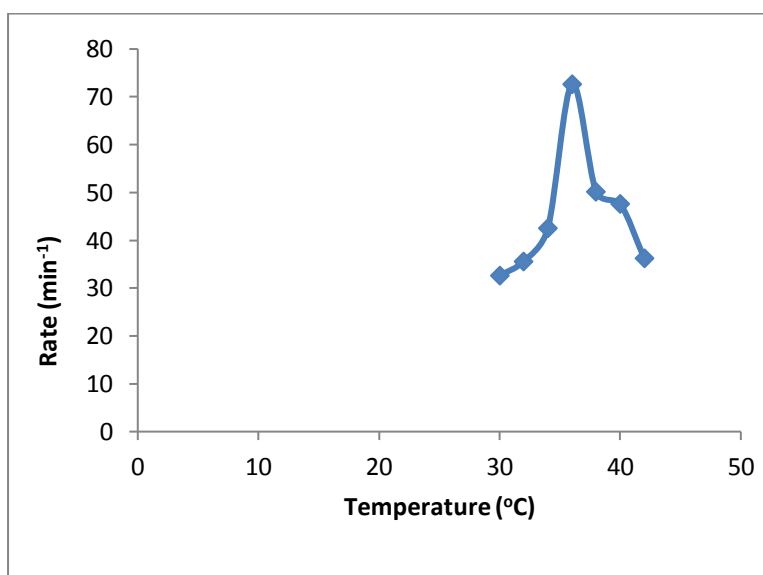


Fig.5: Variation of rate of fermentation of soursop juice with temperature of substrate using 50%(v/v) substrate, yeast 1.0 %(w/v), and pH 5.0

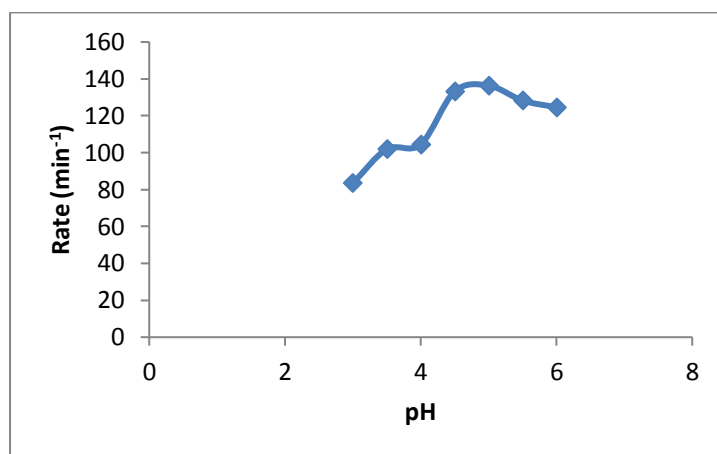


Fig.6: Variation of rate of fermentation of soursop juice with pH of substrate using 50%(v/v) substrate, 1.0 % yeast(w/v), at 30°C

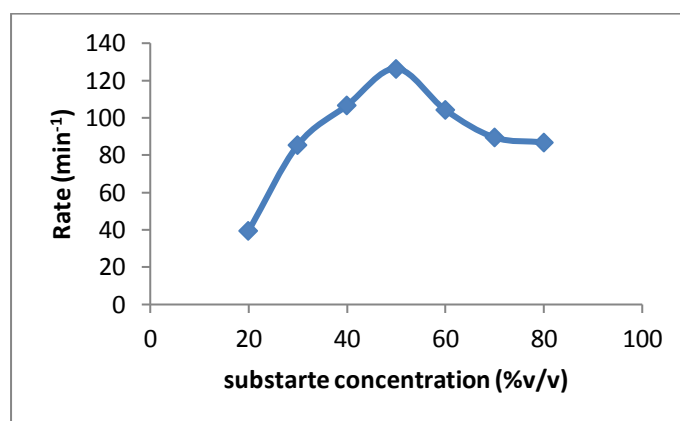


Fig.7: Variation of rate of fermentation of soursop juice with substrate concentration using 1.0% (w/v) yeast, at 30°C and pH 5.0

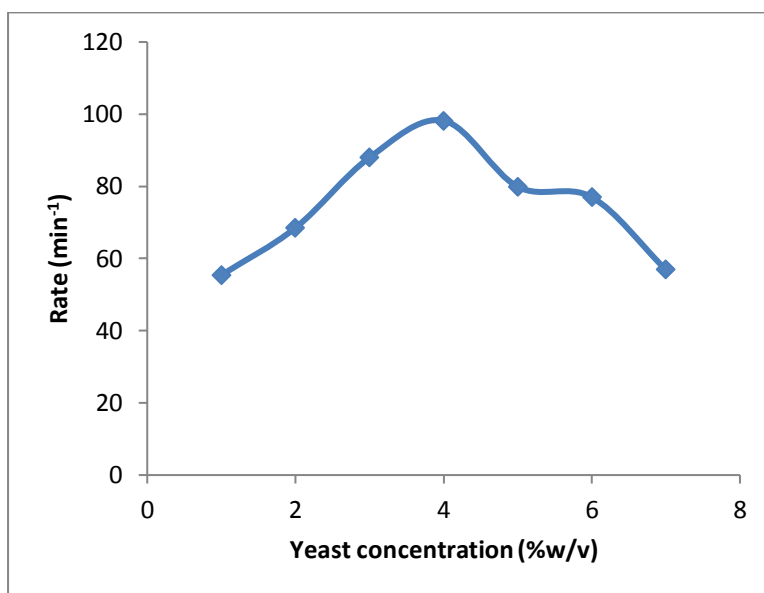


Fig.8: Variation of rate of fermentation of soursop juice with yeast concentration % (w/v) using 50 % (v/v) substrate, at 30°C, and pH 5.0

Table 6: Effect of substrate concentration on the rate, log-rate, log-substrate, reciprocal-rate and substrate of fermentation of soursop juice using 1.0% (w/v) yeast, at 30°C and pH 5.0

Substrate concentration	20	30	40	50	60	70	80
Rate	39.5	85.5	106.7	126.4	104.2	89.5	86.9
Log-Rate	1.596	1.931	2.028	2.101	2.017	1.951	1.939
Log-substrate	1.301	1.477	1.602	1.698	1.778	1.845	1.903
1/Rate	0.025	0.011	0.009	0.007	0.009	0.011	0.011
1/Substrate	0.051	0.033	0.025	0.020	0.016	0.014	0.012

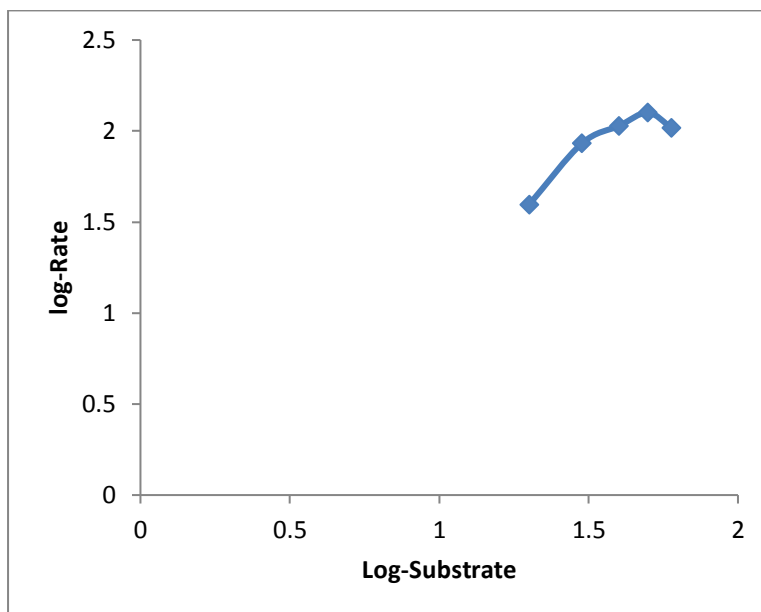


Fig.9: Variation of log-rate of fermentation of soursop juice with log-substrate concentrations using yeast 1.0%(w/v), at 30°C and pH 5.0

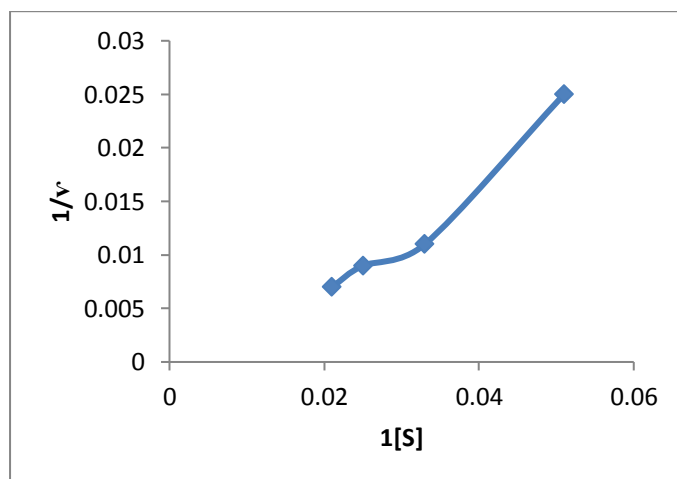


Fig.10: Variation of reciprocal-rate of fermentation of soursop juice with reciprocal-substrate concentrations using yeast 1.0%(w/v), at 30°C and pH 5.0



Table 7: Values of K and n for the Fermentation of Soursop Sugar Using *Saccharomyces Cerevisiae*

	RATE CONSTANT, k	ORDER OF REACTION, n
TEMPERATURE	2.42	Nil
SUBSTRATE CONCENTRATION	1.79	1.16 (1 st order)
YEAST CONCENTRATION	1.58	Nil
Ph	1.84	Nil

Table 8: Kinetic Parameters for the Fermentation of Soursop Sugar Using *Saccharomyces Cerevisiae*

PARAMETER	VALUE
MAXIMUM RATE, V_{MAX} (Mmin ⁻¹)	2.58×10^2
CATALYTIC CONSTANT, k_2 (min ⁻¹)	8×10^{-2}
DISSOCIATION CONSTANT, k_s	1.64×10^2
MICHAELIS CONSTANT, k_m (M)	1.64×10^2
SPECIFIC ACTIVITY (S.CEREVISIAE)	1×10^{-1}

Table 9: Data of the Variation of Volume of CO₂ Produced with time at Different Succinic Acid Concentration of Soursop Juice Using 1.0(W/V) Yeast, At 30°C and pH 5.0

SUCCINIC ACID (mmol l ⁻¹)							
Substrate concentration%(v/v)							
Volume of CO ₂ produced (cm ³)							
	1.0	2.0	3.0	4.0	5.0	6.0	7.0
Time(min)	20	30	40	50	60	70	80
30	1.0±1.3	1.0±1.6	1.0±1.4	1.0±1.0	1.0±1.9	1.0±2.0	1.0±2.3
60	1.0±0.4	1.0±0.3	3.0±1.9	1.0±0.5	1.3±2.1	1.0±0.5	1.0±0.3
90	1.1±1.6	1.0±1.3	5.0±1.8	1.6±1.6	1.6±2.3	1.0±1.5	1.0±0.4
120	1.6±2.0	2.0±1.5	6.0±1.8	2.3±0.9	2.0±1.5	1.0±0.5	1.0±1.9
150	3.1±2.0	2.3±1.4	5.0±0.3	2.3±0.3	2.3±1.3	1.0±1.4	1.0±0.4
180	2.6±2.0	1.3±2.0	4.0±0.4	1.0±1.3	1.5±0.3	1.0±0.4	1.0±1.1
210	1.6±1.9	1.0±20.	2.0±0.6	1.0±0.3	1.0±0.3	1.0±0.4	1.0±0.4

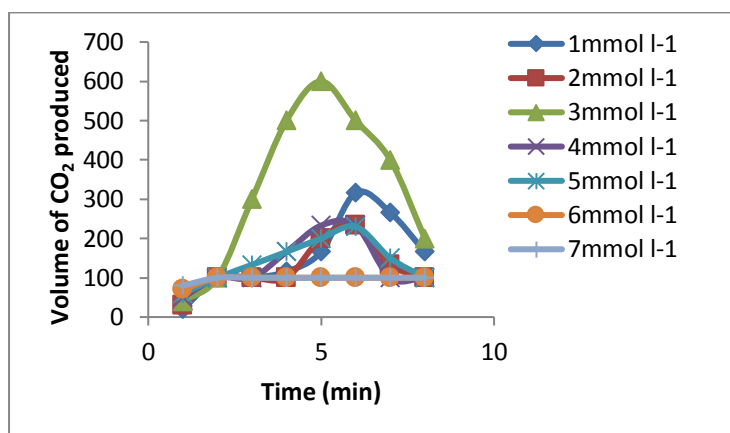


Fig.11: Fermentation of soursop- variation of volume of co₂ production with time at different succinic acid concentrations using 1.0% (w/v) yeast, at 30°C and pH 5.0

Table: 10: Variation of rate of fermentation of soursop juice with inhibitor concentrations using yeast 1.0%(w/v), at 30°C and pH 5.0

CONCENTRATION OF INHIBITOR (mmol l ⁻¹)							
RATE OF FERMENTATION(mol l ⁻¹)							
INHIBITOR	1.0	2.0	3.0	4.0	5.0	6.0	7.0
SUCCINIC ACID	13.7	39.5	30.2	10.1	9.71	2.50	1.66

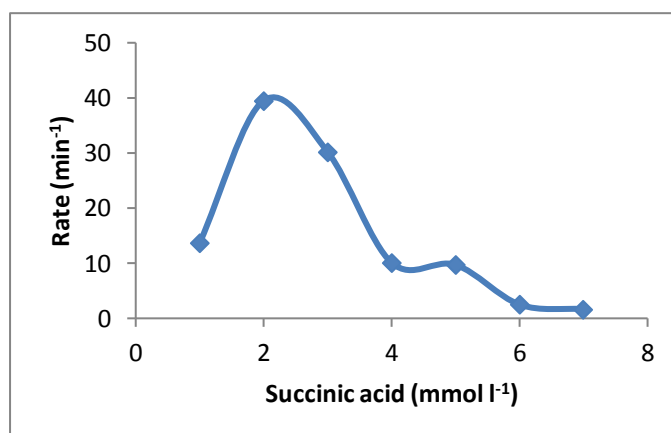


Fig.12: Fermentation of soursop juice - variation of rate of fermentation with succinic acid concentration using yeast 1.0% (w/v), at 30°C and pH 5.0



Table: 11: Variation of log-rate of fermentation of soursop juice with log-inhibitor concentrations using yeast 1.0%(w/v), at 30°C and pH 5.0

LOG -CONCENTRATION OF INHIBITOR(mmol l^{-1})							
LOG-RATE OF FERMENTATION(mol l^{-1})							
INHIBITOR	0.00	0.30	0.40	0.60	0.69	0.77	0.84
SUCCINIC ACID	1.13	1.59	1.48	1.00	0.98	0.39	0.22

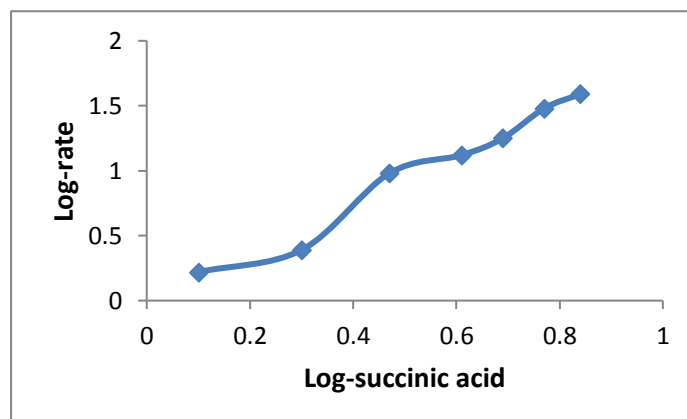


Fig.13: Variation of log-rate of fermentation of soursop juice with log-succinic acid concentrations using yeast 1.0%(w/v), at 30°C and pH 5.0

Table: 12: Variation of rate of fermentation of sugarcane juice with concentrations of the various inhibitor

RATE OF FERMENTATION(units per minute)		
Substrate conc. (mmol l^{-1})	Uninhibited	Inhibited Succinic acid $1-7 \text{ mmol l}^{-1}$
20	102.8	13.7
30	103.9	39.5
40	135.7	30.2
50	148.1	10.1
60	138.3	9.71
70	98.2	2.50
80	42.4	1.66

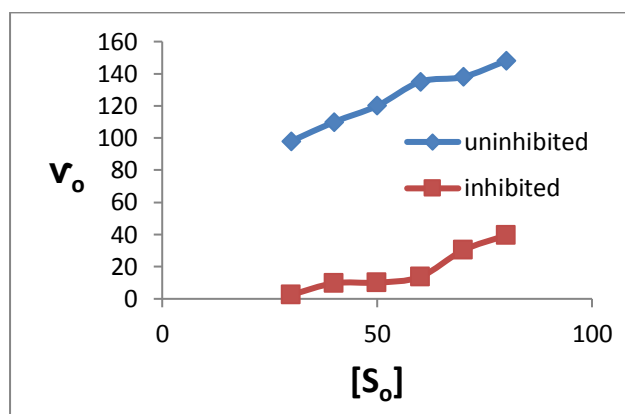


Fig.14: Comparative plot of rate of fermentation of soursop juice with reciprocal succinic acid concentration and uninhibited reactions using Michaelis-Menten Equation

Table: 14: Calculated values of maximum rate and michaelis constant for the fermentation of soursop juice using *saccharomyces cerevisiae* in the presence of inhibitors

PARAMETER	MAXIMUM RATE, V_{MAX} (Mmin ⁻¹)	CATALYTIC CONSTANT, k_2 (min ⁻¹)	DISSOCIATION CONSTANT, k_s	MICHAELIS CONSTANT, k_m (M)	SPECIFIC ACTIVITY (S.C.)
SUBSTRATE	2.58	0.08	1.64	1.64	0.10
SUCCINIC ACID	1.00	0.06	0.24	0.25	0.02

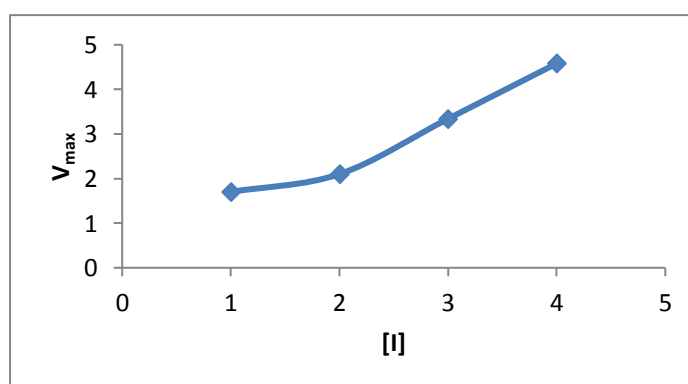


Fig.16: Fermentation of soursop juice - lineweaver-burk plot showing the effect of inhibitors on michaelis constant

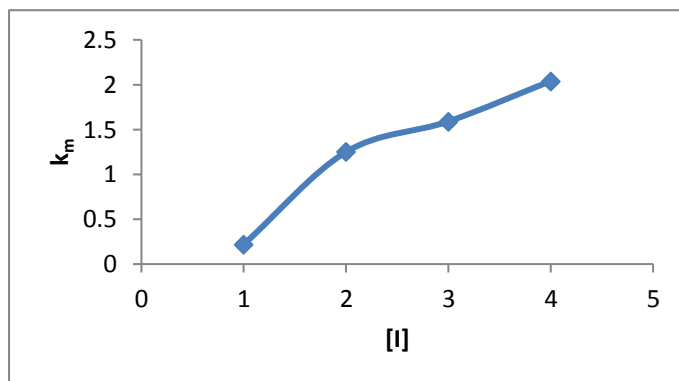


Fig.17: Fermentation of soursop juice - lineweaver-burk plot showing the effect of inhibitors on michaelis constant

Table 15: Calculated values of maximum rate and michaelis constant for the fermentation of soursop juice using *saccharomyces cerevisiae* in the presence of inhibitors

INHIBITORS	$1/V_{max}$	$1/K_m$	K_m/V_{max}
SUCCINIC ACID	0.01	4.00	0.25

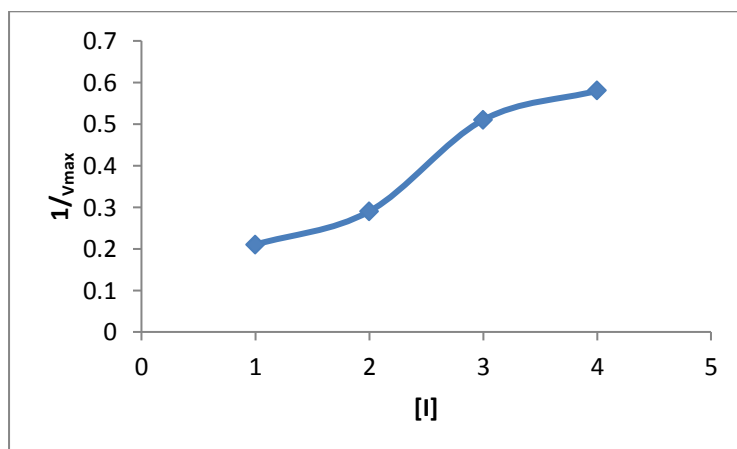


Fig.18: Fermentation of Soursop Juice -Lineweaver-Burk Plot Showing the Effect of Inhibitors on Reciprocal-Maximum Rate

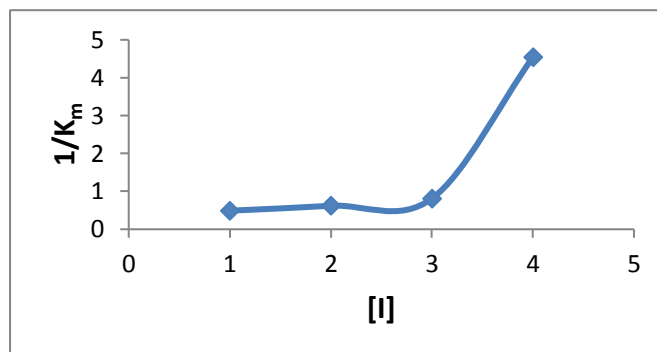


Fig.19: Fermentation of soursop juice - Lineweaver-burk plot showing the effect of inhibitors on reciprocal-michaelis constant

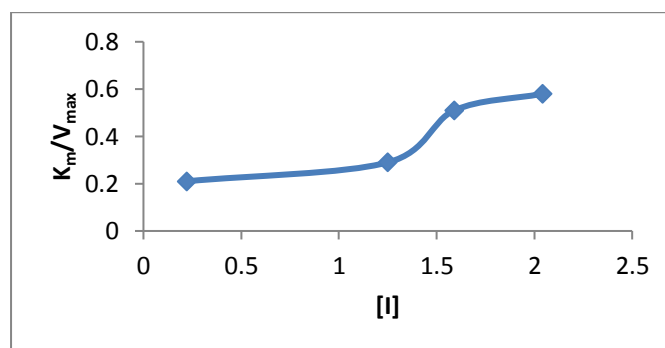


Fig.20: Fermentation of soursop juice - lineweaver-burk plot showing the effect of inhibitors on reciprocal-michaelis constant and maximum rate

Table16: Calculated values of rate constant and order of reaction for the fermentation of soursop juice using *saccharomyces cerevisiae* in the presence of inhibitor

INHIBITORS	Rate constant k	Order of Reaction n
SODIUM BENZOATE	1.48	1.17



DISCUSSION

It was observed that the rate of production of CO_2 increased up to 36°C in substrate and then decreased. The result show that though there was a wide range of temperature over which the yeast enzyme was active, there was a narrow range of temperature ($35\text{--}36^\circ\text{C}$) over which its activity was at maximum. The effect of substrate concentration on rate of fermentation of soursop juice varied in proportion with substrate concentration up to $50\%(\text{v/v})$. Further increase in the substrate concentration showed a decline on the rate of fermentation. This suggests that at the initial stage of the reaction, all active sites of the yeast (enzyme) were saturated by the substrate and further increase in substrate concentration could not lead to increase in the rate of fermentation. The effect of succinic acid concentration on the rate of fermentation varied in proportion with substrate concentration up to $40\%\text{v/v}$ and further increase in succinic acid concentration lead to no increase on the rate of fermentation. This suggests that all the active sites of the yeast were saturated by the succinic acid at the initial stage and further increase in succinic acid could not lead to increase in rate of fermentation. The effect of yeast concentration on the rate of fermentation is seen that there is a wide range of concentration over which the yeast (enzyme) is active and also a narrow range over which the activity is at maximum. It is considered that at high yeast concentration, the substrate becomes unavailable for the large population of yeast for a particular enzyme-substrate system. This suggests that there is a fixed or particular amount of substrate that can be complexed with the yeast. The kinetic parameters: overall rate constant, k , and order of initial reaction, n . The overall rate constant k , with respect to substrate concentration is 1.79; yeast concentration 1.58 and pH 1.84 respectively. The order of initial reaction is first order. The overall rate constant, k with respect to succinic acid, 1.09×10^2 . The order of initial reaction is first order. Most importantly, the kinetic parameters highly valued in fermentation process are: maximum rate of fermentation, V_{max} , catalytic constant, k_2 also known as the turnover number, dissociation constant for the enzyme-substrate complex k_s , the Michaelis constant, k_m , and the specific activity of the enzyme.

The maximum rate of fermentation for substrate V_{max} ; $2.58 \times 10^2 \text{Mmin}^{-1}$ and for succinic acid, $1.00 \times 10^2 \text{Mmin}^{-1}$. The value represents the maximum velocity attainable. It was observed that the maximum rate at which all the enzyme molecules were in the complex form was high and became lower upon the addition of the additive, suggesting inhibition of the fermentation process by the additive due to decrease in the maximum activity of the enzyme.

The catalytic constant, k_2 . The values are $8 \times 10^{-2} \text{min}^{-1}$ and succinic acid, $6.0 \times 10^{-2} \text{min}^{-1}$ which indicates the number of substrate molecules converted into products per unit time. The dissociation constants of enzyme-substrate complexes, k_s for soursop is 1.64×10^2 ; while for succinic acid, 2.40×10^{-1} . There was no significant difference in the values calculated for Michaelis constant and dissociation constant suggesting that the rate of formation of the enzyme-substrate complex was higher than the rate of its dissociation. The Michaelis constant, k_m obtained for soursop is $64 \times 10^2 \text{M}$ and succinic acid, 2.5×10^{-1} is



usually deployed to characterize a particular enzyme-substrate system. K_m is equal to the concentration of substrate required to give half the maximum velocity. The value of k_m in this investigation is quite large with reference to known k_m values that lie between 10^{-1} and $10^{-6}M$ (Chang, 2005). The large value indicates that the binding between enzyme and substrate is very weak with respect to substrate and *Saccharomyces cerevisiae* [E]. The presence of the additive increased the affinity of the enzyme for the substrate making product formation difficult. The specific activity which is the unit of enzyme activity per gram of protein (yeast) is found to be 1×10^{-1} in substrate. The specific activity of enzyme for the addition of succinic acid, 1×10^{-2} . A unit of enzyme is taken to be the amount which will catalyze the reaction of a unit of substrate per minute. Thus, specific activity in relation to enzymes is the ratio of enzyme activity to the total weight of enzyme present in the mixture. This was obtained by cross plotting the rates of fermentation with respect to enzyme and yeast concentrations.

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